Chapter 1

Introduction and Literature Review

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INTRODUCTION AND LITERATURE REVIEW

1.1. INTRODUCTION

Amino acids, are the fundamental building blocks of proteins, offer unique advantages in the synthesis of biodegradable and biocompatible polymers. Amino acids allow for the design of a wide variety of nanoparticles (NPs) that can encapsulate and deliver the drugs effectively by minimizing the adverse effects.[1] Amino acid-based copolymeric NPs are composed of synthetic polymers derived from amino acids, such as poly(L-amino acids) or poly(α-amino acids). These NPs can be engineered to possess specific properties, such as particle size, charge, and hydrophobicity, which enable them to interact with cells and tissues in a targeted manner.[2]

One notable application of poly(amino acid)-polyester graft copolymer NPs is the controlled release of biomolecules. These NPs have been shown to encapsulate wide ranges of drugs/ cytokines/ cells or biomarkers, and release them based on their microenvironments and desired sites of action. This targeted release mechanism enhances therapeutic efficacy while reducing systemic side effects, which is a crucial consideration in cancer treatment.[3] Further, the ability to form stable aggregates with average diameters ranging from 20 to 200 nm under physiological conditions is another significant advantage of amino acid-based NPs. Researchers confirmed that these NPs maintain stability at pH levels typical of human tissues (~6.9), making them suitable for practical applications in clinical settings.[4] The incorporation of amphiphilic block copolymers facilitates drug solubilization and stability, allowing for easy crossing of biological barriers with effective targeted delivery.[5, 6]

Furthermore, the presence of functional groups in amino acid-based polymers offer several key advantages. Firstly, these polymers can be modified to introduce new functionalities such as imaging, molecular targeting, drug conjugation, and enhancing their applicability in biomedical fields.[7] Secondly, they allow for the modulation of swelling and rheological behavior, which can be tailored for specific purposes.[8] Thirdly, they exhibit improved biological properties, including enhanced cell proliferation, adhesion, and biodegradation, making them suitable for tissue engineering and regenerative medicine.[9] Fourth, tailoring the responsiveness of acid-derived polymers by various chemical, physical, and biochemical stimuli, including gas, redox conditions, metal ions, pH, temperature, light, enzymes, glucose, proteins, nucleic acids, and DNA or their combinations thereof help the scientists to develop highly innovative materials for biomedical applications like microfluidic devices, diagnostic kits and biosensors etc.[10] Additionally, these polymers can enhance thermal and mechanical properties, ensuring that they maintain structural integrity under physiological conditions.[11] Finally, the degradation products of these polymers are non-toxic and resemble readily metabolized products from the body, which is essential for minimizing potential side effects and ensuring biocompatibility in medical applications. Overall, the functionalities of amino acid-based polymers place them as a valuable biomaterial in the development and advancement of healthcare therapeutics. The advantage and tremendous applications of amino acid-based polymers motivated us to develop amino acid-based copolymeric NPs for two different applications, i.e., (i) Organ targeting drug delivery systems (OTDDS) and (ii) Tissue regeneration.

Targeted delivery is a pivotal aspect of enhancing therapeutic outcomes. By modifying the surface properties such as incorporating antibodies, peptides, or aptamers over these NPs, researchers can improve their affinity for specific tissues or cells. For example, NPs conjugated with antibodies against the transferrin receptor have been shown to target the brain,[12] while

those conjugated with antibodies against the epidermal growth factor receptor have been shown to target cancer cells.[13] Such strategies not only enhance drug accumulation at the target sites but also minimize off-target effects, thereby increasing the overall therapeutic index. The stealth properties conferred by PEGylation further reduce protein adsorption and prolong circulation time in the bloodstream, enabling more effective targeting of diseased tissues.[14, 15]

In addition to their applications in targeted drug delivery, amino acid-based copolymeric NPs are also promising candidates in tissue engineering and regenerative medicine. These NPs can be loaded with growth factors, cytokines, or other signaling molecules that promote cell proliferation and differentiation. For example, NPs loaded with vascular endothelial growth factor (VEGF) have been shown to promote angiogenesis,[16] while those loaded with bone morphogenetic protein-2 (BMP-2) have been shown to promote bone regeneration.[17] Their biocompatibility ensure that they do not elicit adverse immune responses when used as scaffolds for cell growth and tissue regeneration. The ability to control the release of growth factors or cytokines from these NPs enhances their utility in promoting tissue repair at injury sites. Studies have shown that poly(amino acid) block copolymers can be synthesized with varying side chains to tailor their interactions with drugs and biological environments for advancement in wound healing and tissue regeneration.[18]

Despite the promising results obtained with amino acid-based copolymeric NPs, further research is needed to realize their potential fully. One of the major challenges in the development of these NPs is the need to optimize their design and composition to achieve efficient targeting and delivery of therapeutic agents. This requires a thorough understanding of the interactions between the NPs and cells or tissues, as well as the development of new targeting ligands and surface modification strategies. Another challenge is the need to ensure their safety and biocompatibility. Although these NPs are generally considered to be

biocompatible and biodegradable, further extended research is needed to fully understand their potential toxicity and interactions with cells and tissues.[9, 19]

The development of amino acid-based copolymeric NPs marks a promising advancement in drug delivery systems and tissue regeneration strategies. Their unique properties facilitate targeted delivery, controlled release, and biocompatibility, making them highly suitable for addressing a variety of medical conditions, particularly cancer and regenerative diseases. Ongoing research aimed at optimizing their synthesis and functionalization which is essential for successful translation into clinical practice. As this field progresses, amino acid-based copolymers are poised to play a crucial role in overcoming existing challenges in nanomedicine, offering innovative solutions. The continuous exploration of these NPs holds significant potential for the future of medicine, particularly in enhancing treatment efficacy while ensuring patient safety through targeted delivery mechanisms.

1.2. LITERATURE REVIEW

1.2.1. Polymers and Polymer NPs

A polymer is a large molecule made up of repeating structural units known as monomers, which are linked together by covalent bonds. These macromolecules exist in natural forms, such as proteins and DNA, and synthetic varieties, like plastics. Polymeric NPs are particles that range in size from 1 to 1000 nanometers and can be loaded with active compounds that are either encapsulated within or adsorbed onto the polymeric core. Their versatility enables them to play vital roles in medicine, engineering, and materials science.

1.2.1.1. Amino acids and their bio functions

Amino acids are organic compounds that contain both amine ($-NH_2$) and carboxylic acid (-COOH) functional groups. When polymerized, they serve as the building blocks of proteins, which are essential for the synthesis and construction of enzymes, hormones and

neurotransmitters etc. They participate in numerous physiological processes, including muscle growth, digestion, respiration, cellular processes, metabolism and immune functions. For instance, Glycine acts as an inhibitory neurotransmitter in the central nervous system, regulating neuronal excitability and influencing behaviors such as sleep and appetite. Additionally, it is essential for collagen (type I collagen) synthesis, which provides tensile strength during tissue repair.[20] Phenylalanine serves as a precursor to tyrosine, which is further converted into neurotransmitters such as dopamine, norepinephrine, and epinephrine—key players in mood regulation and stress response. Leucine promotes protein synthesis and lowers glucose levels in diabetes, histidine is involved in the enzymatic process, cysteine acts as an antioxidant while tryptophan is involved in serotonin production, etc. A deficiency in amino acids can lead to health issues such as weakened immunity, impaired growth and mood disorders.[21] Further, most studies have indicated that L-type chiral amino acids activate immune responses and signaling pathways, while D-type chiral polymers enhance cell adhesion, promote macrophage accumulation, and exhibit anti-inflammatory properties.[22] In pharmaceutical industries, chirality is a critical factor since the enantiomers of chiral drugs can exhibit different pharmacokinetic and pharmacological behaviors. Utilizing pure enantiomers instead of racemates can improve both the effectiveness and safety of treatments.[23]

1.2.1.2. Versatility of amino acids-based copolymer NPs based on their physicochemical properties

Based on the architecture, polymeric NPs can be categorized into various types, including micelles, nanospheres, polyplexes, nanocapsules, nanogels, and dendrimers.[24] The presence of oil in the nanocapsules leads to a vesicular structure while the absence of oil leads the polymeric chain to a sphere shape. At critical micelle concentration (CMC), amphiphilic polymers get aggregated and lead to form a lipophilic core with a hydrophilic shell termed as

‘micelle’. The shape of the micelle is not limited to a sphere; it can be synthesized in an elongated form also, which exhibits enhanced rheological and viscoelastic properties.[25, 26] Further, although the dendrimers, a three-dimensional, mono-dispersed, globular structure offer high loading capacity with enhanced gene delivery, however its size, toxicity, and bio-distribution issues limit its use in healthcare therapeutics.[27]

The versatility of amino acid-based polymers is significantly influenced by various factors, such as shape, size, charge, functional groups, stability, aspect ratio, etc. The shape of a polymer can greatly influence its behavior and interactions in biological environments. For instance, spherical or globular shapes are often advantageous for drug delivery applications as they can facilitate cellular uptake through endocytosis. In contrast, elongated or fibrous shapes can enhance mechanical strength and provide structural support in tissue engineering applications. The shape can also affect the surface area-to-volume ratio, influencing the rate of drug release and interaction with cellular membranes. For example, NPs with a spherical shape may provide a higher surface area for drug loading compared to larger, irregularly shaped particles.[28, 29] Further, size influences the pharmacokinetics of the polymer; smaller particles may exhibit faster circulation times and improved bio-distribution compared to larger ones; for example, particles <8 nm are filtered out by kidneys, while those between 10 -20 nm can cross the blood-brain barrier and possess imaging capabilities. Particles ranging from 20-100 nm can readily bypass physiological barriers and have a high potential for circulation, whereas particles between 200 nm to 1 μm are typically cleared by the spleen.[30] Furthermore, the charge of these polymers plays a pivotal role in their interactions with other biomolecules and cells. Polymers can be cationic, anionic, or neutral depending on the amino acids used in their composition. Cationic polymers often exhibit enhanced cellular uptake due to electrostatic interactions with negatively charged cell membranes. This property makes them particularly useful for gene delivery applications, where they can facilitate the transport of nucleic acids

into cells. Conversely, anionic polymers may be favoured for specific applications, where repulsion from negatively charged surfaces is beneficial, such as in certain drug delivery systems that require controlled release mechanisms.[31] The presence of multiple functional groups in amino acids like -COOH, -NH_2, -SH, -OH, -F, etc. allow for multiple conjugations or different chemical and surface modifications such as stimuli responsiveness and specific targeting capabilities. For example, incorporating hydrophobic functional groups can enhance the polymer's ability to encapsulate hydrophobic drugs, while hydrophilic groups can improve solubility and biocompatibility.[8, 11] The aspect ratio (length-to-width ratio) of polymer structures significantly impacts their performance in various applications. High aspect ratio materials, such as fibers or nanotubes derived from amino acids, can provide enhanced mechanical properties and greater surface area for interactions compared to low aspect ratio materials like spheres or films. In tissue engineering, high aspect ratio scaffolds can mimic natural extracellular matrices more effectively, promoting cell adhesion and growth. Moreover, elongated structures may facilitate directional transport within biological systems or create anisotropic properties that are beneficial in specific applications.[32] Thus, by manipulating these properties through careful design and synthesis strategies, researchers can develop advanced biomaterials tailored to specific therapeutic needs.

1.2.1.3. Functional poly(amino acid) copolymer NPs and their synthesis strategies

Poly(amino acids) such as poly(aspartic acid), poly(lysine), poly-γ-(glutamic acid), and poly(arginine) are naturally occurring polymers essential for biological processes formed by linking amino acids through amide bonds. In contrast, polymers designed by linking amino acids with non-amide bonds are referred as pseudo-poly(amino acids). These pseudo-polymers can adopt various architectures, including block, branched, hyperbranched, or dendritic structures, which enhance their mechanical properties and other characteristics.[33] To prepare

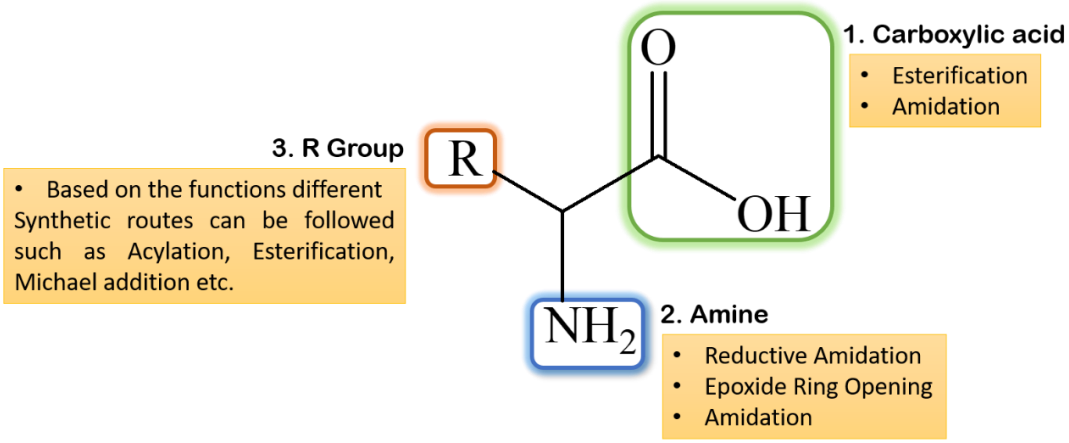

***Figure 1.1**. Synthetic strategies of functional amino acid polymers.*

amino acid-based polymers, different synthetic strategies are employed based on the functional groups present, as shown in **Figure 1.1**.

Different techniques followed for the synthesis of poly(amino acid) based copolymer NPs are Mini emulsion free radical polymerization[34], Ring-Opening Polymerization (ROP)[35], Reversible Addition-Fragmentation Chain Transfer (RAFT) Polymerization[36], Step-Growth Polymerization, Thermal or Chemical Conversion, Post-Polymerization Modifications[37] and Ionic polymerization of monomers[38], etc. Free radical polymerization is a widely used method for synthesizing polymers from vinyl monomers through a chain reaction mechanism. This process consists of three main stages: initiation, propagation, and termination. Common initiators for this process include azo compounds and peroxides, making it a versatile technique for producing a variety of polymers and materials. ROP involves the polymerization of N-carboxy anhydrides (NCAs) derived from amino acids, which allows for the creation of well-defined poly(amino acid)s, including block and random copolymers, by controlling the sequence and addition of NCAs to an initiator. This technique is highly versatile and enables the formation of various architectures due to its living polymerization nature. RAFT is employed to synthesize amino acid-based block copolymers with specific properties, such as stimuli responsiveness (pH, thermal). This method allows for precise control over molecular

weight and composition, facilitating the creation of amphiphilic and double-hydrophilic block copolymers. However, material obtained through this method possesses specific challenges such as compatibility of RAFT agents with different monomers, complexity in the synthesis of RAFT agent and its stability, oxygen sensitivity and their rate of retardation in polymerization process etc. Most importantly presence of sulfur-containing end groups may show some undesirable effects in the certain applications which might require additional purification steps in the end product.[39, 40]

Further, amino acids can undergo step-growth reactions which is a straightforward method to yield homopolymers or copolymers based on the amino acids selected. After initial polymer synthesis, multiple modifications can introduce new functionalities to the side chains, enhancing their properties for specific applications, which may include atom transfer radical polymerization (ATRP)[41], ring-opening metathesis polymerization (ROMP)[42], metal-catalyzed metathesis or insertion polymerization technique[43] and nitroxide-mediated polymerization (NMP).[44] The polymerization of monomers derived from amino acids, such as N-carboxy α-amino acid anhydrides, can also be used to create copolymers which consist of a randomized mixture of synthetic polypeptides.

1.2.1.4. Advantages of amino acid-based copolymers over amino acid-based homopolymers

Homopolymers composed of a single repeated unit. Amino acid-based homopolymers, lack functional group diversity and limit their potential use in biomedical applications, such as tissue engineering and microfluidic devices, etc.[45, 46] Their poor solubility in aqueous solutions makes them difficult to process and use for diagnostic and therapeutic purposes.[8] They can stimulate an immune response, leading to the production of antibodies and potential inflammation.[47] Amino acid-based copolymers offer several advantages over amino acid-

based homopolymers, making them particularly valuable in various applications such as (i) Enhanced Functional Diversity: copolymers can incorporate different types of amino acids, allowing for a broader range of chemical properties and functionalities compared to homopolymers, which consist of a single type of amino acid. This diversity enables tailored interactions with biological systems and improved performance in drug delivery applications;[18] (ii) Amphiphilic Properties: many amino acid-based copolymers exhibit amphiphilic characteristics, meaning they contain both hydrophilic and hydrophobic segments. This property facilitates self-assembly into structures such as micelles and NPs, where the hydrophobic core acts as a reservoir with effective cavities that encapsulate hydrophobic drugs through strong hydrophobic interactions, thereby enhancing drug loading capacity. Meanwhile, the hydrophilic shell sterically stabilizes the micelles, preventing aggregation and opsonization, which improves colloidal stability and prolongs circulation time in the bloodstream. These micelles also exhibit low CMC, reducing dissociation upon dilution in biological fluids and further enhancing stability;[18, 48] (iii) Tunable Degradation Rates: the degradation rates of copolymers can be adjusted by varying the composition of the amino acids used. This tunability allows for the controlled release of therapeutic agents at the target site, optimizing treatment efficacy while minimizing side effects;[18] and (iv) Biocompatibility and Safety: like homopolymers, amino acid-based copolymers are generally biocompatible since they are derived from natural amino acids. However, their ability to incorporate various functional groups can further enhance their safety profile and reduce immunogenicity.[49] Whereas, introducing one or more different amino acids as mer units in the homopolymer chain makes them copolymers. Subsequently, this copolymer system provides scientists with an option to develop more versatile biomaterials with improved functional properties. Based on these factors, it is hypothesized that synthesizing an amphiphilic block copolymer of glycine and

phenylalanine could effectively address key challenges associated with block copolymer drug delivery systems.

1.2.1.5. Biomedical applications of polymers/poly(amino acid)s NPs

Poly(amino acid) copolymers are gaining prominence in biomedical applications, especially for drug delivery, because of their biocompatibility, enzyme degradability, unique structures, and stimuli responsiveness. Simultaneously they are used for wound care management, tissue regeneration, orthopedic implants, scaffold design, and infectious diseases.

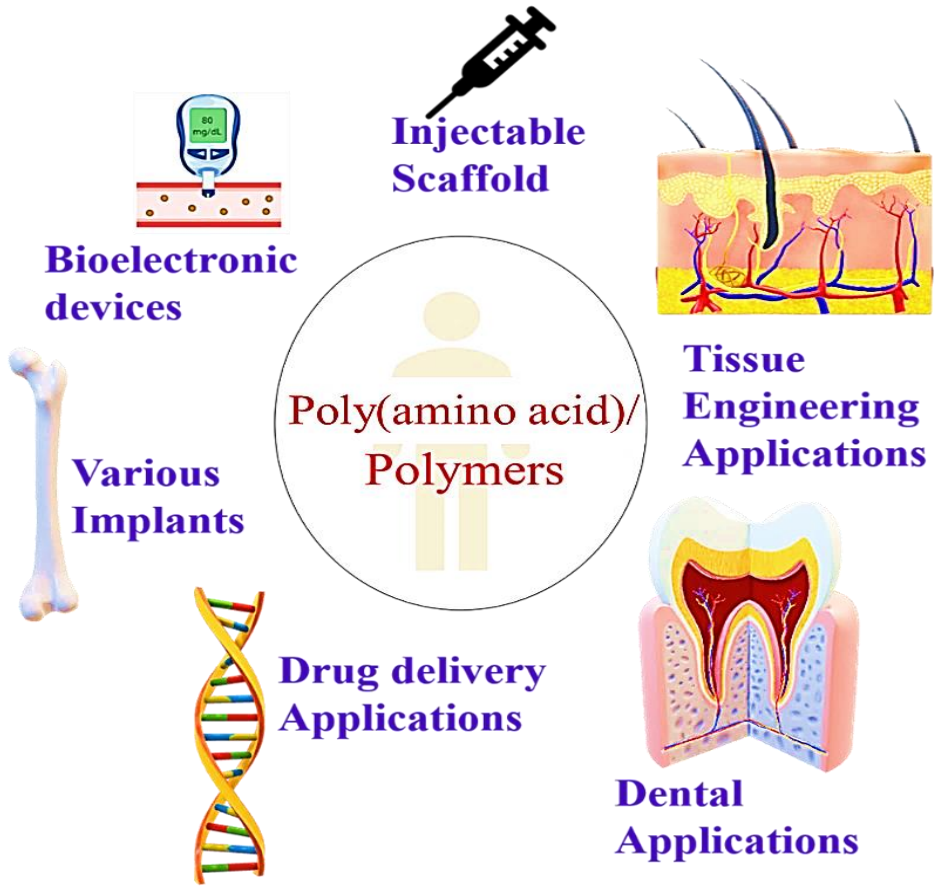

***Figure 1.2**. Represents biomedical applications of polymers/poly(amino acid) NPs.[50]*

A table has been attached below with the list of Poly(amino acid)s used for various biomedical applications (**Table 1.1**).

Table 1.1. List of Poly (amino acid)s used for various biomedical applications.

	Poly(amino acid)s used	The effective area of applications	Reference
Wound Management			
Sutures	PCL, PLA, PGA, PLGA	Tissue repair and improve wound healing, Effective *In vivo* degradation	[51, 52] [53] [54]
Wound healing Dressing Material	Cellulose, dextran, & (1→3)-β-D-glucans, Chitin, Chitosan, PEG/PEO, PLA, Collagen, Gelatin, Elastin, Fibrin and SF, PLGA/PEG, PCL, poly(N-acryloyl glycine) and nitric oxide loaded poly(N-acryloyl glycine) NPs, micelle made up of arginine, phenylalanine and lysine	Delivery approaches in diabetic conditions, antimicrobial properties with less cytotoxic effect, Self-powered hand-held gun for direct dressing, efficient *in vivo* wound healing, inhibit MRSA infection in *vivo*	[55] [56] [57] [58-61]
Adhesives	Chitosan and modified Chitosan	Tissue sealing properties, Act as a hemostat, Drug delivery vehicle for therapeutic proteins and peptides	[62]
Drug delivery systems			
Conjugates	Alginate with a sulfate group; Alginate-calcium carbonate incorporated with Paclitaxel	Trapped greater quality of drug; Prepared by Co-precipitation method	[63]-[64]
Tumor targeted	PEGylated ICG-encapsulated NPs; Doxorubicin loaded GSH tagged CQDS; immunomodulatory, anti-inflammatory, antioxidant, internalization-promoting and apoptosis-promoting functions of poly(amino acid)s, PMAG-b-PAA, poly(N-acryloyl amino acids of valine, valine methyl ester, aspartic acid and serine, poly (N-vinylpyrrolidone)-b-poly (N-acryloyl-L-phenylalanine), PEG-b-poly(glutamic acid)-g-PEI	Length of the PEG chain target angiogenesis in the tumor, *in vitro* and *in vivo* tumor targeting capacity with minimal side effects as compared to commercialized anticancer drugs; reduce side effects with improves synergistic effects; cytotoxic effect against MCF-7 and A549; treatment against MRSA; induce toxicity on MCF-7, gene delivery against multiple tumor cells	[65-71]
Oral drug delivery	PGA, (Poly(glycerol-adipate), Starch	Hydrophobic drug delivery, PGA degradation in oral gastric track	[72]
Polymeric microneedle	Poly(vinylpyrrolidone)	Controlled systems for gene delivery	[73]
Scaffold	Gelatin + (Tobramycin/Caspofungin/Vancomycin /Polymyxin B/Amphotericin B/daptomycin)	Primary generation for human dermal fibroblast and for wound healing	[74]

	PLA with Paclitaxel and VEGF	As a carrier for drug delivery	[75]
	PCL with drug Clotrimazole	Shows antifungal activity	[76]
	HA-based Doxorubicin	Drug delivery vehicle against human breast cancer	[77]
Tissue engineering			
Scaffold	Electrospun collagen-chitosan-PU based nanofibrous scaffold; Chitosan/ PCL nanofibrous scaffold; Microporous scaffold of bilayer Chitosan/ Gelatin	Flexible at high tensile strength; Rapid induction and anticoagulation of re-endothelialization Similar to blood vessels in morphology and mechanical properties.	[78],[79],[80]
Patches	Chitosan/heparin multilayered patch	*In vitro* and *In vivo* results showing long-term effects in any substrate	[81]
ligament	Collagen, PLA, Silk, Alginate, HA, Chitosan	Good Mechanical properties, regeneration of damaged tissue, used in the 3D printing area	[82]
	Chitosan/Cellulose nanofibers	Biomaterial to promote intervertebral disc regeneration	[83]
Infectious diseases			
Bacteria	PHB based Calcium–Iron Layered Double Hydroxide nanocomposites with Norfloxacin; PLA/MMT nanocomposite films with gentamicin and neomycin,	Show very high drug release efficiency and effective antibacterial activity against *S. aureus* and *E. coli*, Good antibacterial properties	[84, 85]
Fungus	PCL with drug Clotrimazole scaffold	Antifungal activities	[76]
Malaria	PCL loaded with Chloroquine and DHA (Dihydroartemisinin)	Temperature responsive mesoporous polymeric carrier used to treat malaria	[86]
Eye infection	Collagen/Gelatin/Sodium Alginate liposomal NPs encapsulated with Chloramphenicol	Ophthalmological Infectious disease.	[87]
Foodborne pathogens	Potato starch, Chitosan, Corn starch, Carboxymethyl cellulose, and Arabic gum	Enhancing the antimicrobial activity, and preventing foodborne pathogens	[88]

1.2.1.6. Current clinical and preclinical status of nanomedicines

Over 2,000 nanomedicines are registered for clinical trials globally, among which 409 are based on therapies and diagnostics. Notably, 40% of these trials target cancer treatment. As of 2021, around 100 nanomedicines have received regulatory approval, with a significant number

in various stages of clinical development (563 new nanomedicines). By 2015, the FDA had approved 13 nanomedicines for multiple diseases.[89] Liposomes and protein-based NPs are among the most extensively investigated formulations in clinical trials. Several nanomedicines have been approved for clinical use, including liposomal formulations, such as Doxil and Ambisome, and NP-based formulations, like Abraxane.[90] Ongoing preclinical studies focus on optimizing formulations for enhanced safety and therapeutic efficacy, exploring diverse applications beyond cancer, including infections and metabolic diseases. NPs-based COVID-19 vaccines (e.g., Pfizer-BioNTech and Moderna) have shown high efficacy rates (>90%).[91, 92] NPs-based therapies are being explored for improving drug sensitivity and circumventing resistance mechanisms in cancers. Additionally, there is a growing interest in developing personalized nanomedicines, tailored to individual patient's needs, using techniques like nanoscale formulation and targeted delivery.[93]

1.2.1.7. Rationale for developing a library of organ targeting copolymer nanoparticles

The idea to synthesize copolymer NPs with varying sizes and compositions allows researchers to discover materials with novel properties that can be utilized in advanced biomedical applications. The systematic creation of NPs libraries enables efficient screening for enhanced chemical and physical properties. This approach not only speeds up the discovery process but also allows for identifying NPs with specific desired traits, such as improved catalytic activity or targeted drug delivery capabilities. Further, developing an effective organ-targeted drug delivery system (OTDDS) necessitates a clear understanding of how the physical and chemical properties of OTDDS influence the biological processes at the molecular, cellular, and organ levels.[94] Among various targeting mechanisms, endogenous targeting is emerging as a promising approach based on how the NP's chemical versatilities lead to bind with distinct plasma protein subsets of target organs, by directing NPs to the specified organs and enhancing

cellular uptake.[95, 96] Although the chemical modification can enhance the target specificity of the delivery vehicles, however, they can also increase the toxicity and side effects in the long run.[227] These insights have motivated us to synthesize copolymer NPs with varying ratios (x:y) of the biologically safe monomers (amino acid-based) without using any endogenous or exogenous ligands for enhanced organ-specificity.

1.2.2. Role of Computational Methods in Healthcare Therapeutics

Computational methods play a vital role in healthcare therapeutics and help to transform the way we design, develop, and deliver treatments. It reduces the time and cost associated with the initial set of trial-and-error experiments. The key aspects of computational methods include (i) Drug Discovery and Development, (ii) Personalized Medicine, (iii) Disease Modelling and Simulation, (iv) Pharmacokinetics and Pharmacodynamics study, (iv) Clinical trial design and Optimization, (v) Real World Evidence Analysis and (vi) Artificial Intelligence and Machine Learning, etc.[97, 98] Computational methods help to predict self-assembly structure, drug loading capacity, interaction between biomaterials and biological systems, etc., and address many unresolved questions generated during the development stage.

Network pharmacology and molecular docking are transformative approaches in modern drug discovery, addressing the complexity of diseases and enhancing therapeutic development. Network pharmacology employs a systems biology perspective, analyzing interactions across biological networks to identify multi-target therapies, while molecular docking predicts how molecules bind to target proteins, optimizing drug design. Together, they bridge holistic disease understanding and precise molecular interventions. The integration of network pharmacology with molecular docking further helps scientists to predict off-targets, design new drugs, and identify potential genes responsible for specific diseases.[99-102] Further, Molecular Dynamics (MD) simulation, a crucial computational method allows researchers to model the physical

movements of atoms and molecules over time by providing insights into the dynamic behavior of biomolecules. The arrangement of the polymer chain or RNA sequences can also be performed using MD Simulation.[103-105] Thus, by leveraging computational methods in healthcare therapeutics, biomaterials can be more effective, efficient, and personalized and ultimately lead to better patient outcomes with improved quality of life.

1.2.3. Brief on Triple Negative Breast Cancer (TNBC)

Triple-negative breast cancer (TNBC) is particularly challenging and aggressive subtype of a breast cancer, distinguished by the absence of three key receptors that are commonly present in other forms of the disease: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). As TNBC does not respond to hormonal therapies or HER2-targeted drugs, which are effective for other breast cancer subtypes, chemotherapy acts as the primary treatment option.[106, 107] TNBC is recognized for its aggressive behavior and is characterized by several key features such as (i) high rate of reoccurrence, (ii) rapid growth and spread, (iii) poor prognosis, (iv) heterogeneity, (v) demographic, (vi) prevalence, (vii) drug resistance, and (viii) metastatic pattern, etc.[108-111] Some novel approaches like PARP inhibitors, immunotherapies, and targeted molecular strategies are emerging as promising alternatives.[106] Understanding the molecular characteristics and unique features of TNBC is crucial for developing personalized medicines with better patient outcomes.

1.2.3.1. Global statistics on TNBC

TNBC accounts for 10-20% of all breast cancer types. Some studies suggested that it can be reached to as high as 15-25% by 2029.[108] Breast cancer is the most commonly diagnosed cancer worldwide. According to the World Health Organization (WHO) report, in 2022, there were approximately 2.3 million new cases.[112] Breast cancer alone accounted for over 29%

of all new cancer cases for women in 2022.[113] It is estimated that approximately 1,70,000 cases of breast cancer are TNBC worldwide. The 5-year survival rate for TNBC is approximately 70-77%, while for other breast cancer subtypes, it is around 90%.[109] The median time to death for patients with TNBC is estimated at 4.2 years compared to 6 years for other breast cancers. Survival after a TNBC diagnosis is approximately 10 years at most, in comparison to 18 years for patients with other breast cancers. The risk of distant recurrence is maximized within 1-3 years after surgery and remains steady afterward.[114] TNBC is more common in younger women (under 50 years old), though younger women have increased rates of BRCA (tumor suppressor gene) and basal TNBC and apocrine and neuroendocrine TNBC. The prevalence of TNBC is approximately twice as high in African American women (47%) compared to white women (22%). Further, tumor recurrence is reported to occur at 1.2 years, which is shorter than in non-TNBC patients.[108, 110]

1.2.3.2. Challenges associated with the treatment of TNBC

Several challenges are associated with the treatment of TNBC. In brief, the molecular heterogeneity of TNBC creates a significant barrier to developing targeted therapies, require a more complex approach for the treatment.[114] The high metastatic capability of TNBC results in earlier relapses and distant recurrences,[106] often to vital organs such as the brain, bone, and lungs. Chemotherapy can lead to the enrichment of cancer stem cells, which are associated with disease relapse and metastasis.[109] The toxicity of chemotherapeutic drugs limits the long-term treatment options for TNBC patients.[115] Further, components of the tumor microenvironment (TME), such as transformed extracellular matrix (ECM), soluble factors, and immune suppressive cells, can hamper the antitumor responses. Exosomes, which are nanovesicles, play a critical role in orchestrating the TME by communicating with different cells within it. The tumor can hijack physiological immune responses and cause immune

tolerance. Immunosuppressive cells in the TME can hinder the effectiveness of immunotherapies.[106] Most importantly, predictive biomarkers such as PD-L1 expression, TIL density, tumor mutation burden (TMB), and immune gene signatures need further investigation.[116] It is also challenging to identify changes induced by drug treatment that may be associated with the upregulation of compensatory signaling pathways in drug-resistant cancer cells.[117, 118] Finally, even though new targeted therapies show initial promise, resistance mechanisms can develop, limiting their long-term effectiveness. This requires ongoing research to understand these resistance mechanisms and to develop novel therapeutic approaches to overcome them.

1.2.3.3. Polymers and poly(amino acid)s based nanocarriers developed for TNBC

Polymeric NPs including poly(amino acid)s are gaining popularity as nanocarriers for targeted drug delivery in TNBC due to their stability, easy surface modification, stimuli-responsive and controlled drug release behaviour, ability to condense more than one therapeutic moiety, tumor-specific payload delivery, and enhanced permeation and retention effect. They enhance efficacy, bioavailability, and reduce the toxicity of therapeutic agents. In the context of different amino acid's unique properties glycine's small size and biocompatibility allow for efficient drug encapsulation and controlled release at the target site; the presence of guanidinium groups in arginine promotes efficient cellular uptake through strong interactions with negatively charged cell membranes; hydrophobic aromatic side chain of phenylalanine facilitates the self-assembly of nanostructures with effective encapsulation of hydrophobic drugs; and inherent fluorescence properties of tryptophan allows for improved cellular uptake and real-time tracking of drug delivery in cancer cells, etc. Further, a table has been given below with a list of recent polymeric nanocarriers used for TNBC treatment (**Table 1.2**).

Table 1.2. List of polymers and amino acid based nanocarriers used for TNBC treatment.

Polymer/ amino acid based NPs	Drug(s)	Model used	Reference
Poloxamer 407	Camptothecin	Xenograft mice model	[119]
methyl- poly(ethylene glycol)-PCL	Curcumin	*In vivo*	[120]
PLGA-PVA	Niclosamide and curcumin	*In vitro*	[121]
terpolymer	pDNA	*In vivo*	[122]
Chitosan	Resveratol	*In vitro*	[123]
PLGA	PD L1 siRNA	*In vitro*	[124]
Chitosan coated PLGA	Thymoquinone	*In vitro*	[125]
Poly(N-acryloyl glycine-co-N-acryloyl phenylalanine methyl ester)	Dihydroartemisinin and Piperine	*In vitro*	[126]

1.2.3.4. Potential role of phyto-drugs and their nanoparticle based delivery systems used for TNBC

Phytochemicals represent a diverse array of compounds with a range of biological activities, making them a valuable resource for cancer drug discovery. Traditional medicines and ethnopharmacological data can provide a starting point for identifying promising phytochemical candidates for further study. These compounds can act as anti-proliferative, anti-metastatic, and chemosensitizing agents, by offering a potential strategy to overcome the limitations of conventional TNBC treatments.[112] They can induce genetic, transcriptomic, and proteomic alterations in cancer cells, impacting gene expression and protein function.[106] They can also reduce cancer cell proliferation, migration, invasion, and inhibit tumor growth.[112] Some phytochemicals can modulate the TME, which plays a crucial role in cancer progression, by reducing immune suppression and impacting cell communication within the TME. Curcumin, resveratrol, and EGCG inhibit pathways that cause TNBC. Fisetin and quercetin have been shown to reduce migration and matrix invasion of TNBC cells *in vitro* and TNBC cell metastasis in zebrafish.[127] They also disrupt the activities of several protein kinases in MAPK and STAT pathways. Piperine can enhance the effectiveness of TRAIL-based

treatments by reducing phosphorylation of p65 (part of the NF-κB pathway) and the production of survivin.[109] Further, antimalarial drugs like artemisinin and dihydroartemisinin (DHA) have shown promise in treating TNBC by inducing apoptosis, inhibiting proliferation, metastasis, and modulating key signaling pathways. DHA can enhance the effectiveness of other treatments like TRAIL, and it can induce ferroptosis in cancer cells.[128] Additionally, combining phytochemicals with chemotherapy or immunotherapy can lead to synergistic anticancer effects. For example, combining Berberine with TRAIL or noscapine with docetaxel or doxorubicin improves TNBC treatment.[129] The use of phytodrugs with PARP inhibitors has also gained attention because of their potential to improve efficacy and mitigate drug resistance.[112, 117] Further, the development of NPs based delivery systems for phyto-drugs is important because it overcomes key challenges associated with natural plant compounds, including poor solubility, low bioavailability, and rapid elimination from the body. By encapsulating these compounds within the NPs, their stability is enhanced, controlled and sustained drug release is achieved, and targeted delivery to specific cells or tissues becomes possible.[130, 131] These improvements lead to increased therapeutic effectiveness with fewer side effects, and reduced toxicity compared to the conventional formulations. Additionally, NP platforms allow for customization by loading specific combinations of phyto-drugs or combining them with conventional drugs.[132] This enables the treatments to be precisely tailored according to the molecular and phenotypic traits of an individual's by supporting the goals of precision medicine.[133] Such personalized approaches address tumor heterogeneity, help to overcome drug resistance, and focus on critical cell populations like cancer stem cells, thereby enhancing treatment success. Thus, standalone or combination therapy of phyto drugs could be utilized for the better management of TNBC.

1.2.3.5. Combination therapies in TNBC

Combination therapies for TNBC may involve combining chemotherapy, targeted therapies, or immunotherapy among each other.[134-136] For example, Chemotherapy combinations: anthracyclines and taxanes, a combination of platinum agents or phytodrugs, a cocktail of any TNBC inhibiting drugs, mixture of fluorouracil, doxorubicin/epirubicin, cyclophosphamide, or doxorubicin, cyclophosphamide with paclitaxel or docetaxel; targeted therapy combinations: olaparib and talazoparib, PARP inhibitors with chemo or immune therapy, cetuximab, panitumumab, and erlotinib with chemo drugs, mTOR inhibitor with doxorubicin, ipatasertib and capivasertib with paclitaxel, photothermal therapy (PTT) and photodynamic therapy with PARP inhibitors; Immune therapy combinations: pembrolizumab and atezolizumab with chemotherapy, and anti-PD-1/PD-L1 with anti-CTLA-4 inhibitors. These combination therapies aim to enhance treatment efficacy while potentially reducing the side effects associated with conventional chemotherapy alone.

1.2.3.6. Complications associated with off-target drug delivery and the importance of targeted therapies in TNBC

The complications associated with off-target delivery systems can be listed as unintended side effects, systemic toxicity, an increased dosing frequency, reduced efficacy, immunogenicity, low bioavailability, poor pharmacokinetics and pharmacodynamics, and rapid resistance development etc.[137-143] Further, off-target drug delivery, like chemotherapy, can harm healthy cells also. Therefore, to overcome these complications, targeted delivery systems, such as NPs, liposomes, and conjugates, are being developed to ensure specific and efficient delivery of therapeutic agents to target sites with a minimum number of dosages. Different targeted therapies can be listed as PARP inhibitors, EGFR inhibitors, Folate receptor alpha (FRα), PI3K/AKT/mTOR pathway, tyrosine kinase inhibitors, anti-angiogenic agents,

antibody-drug conjugates, TME, and immune checkpoint inhibitors. Each therapy has its advantages and limitations.[144, 145] Among all receptor targeting approaches (transferrin, androgen, nucleolin, etc.), FRα is emerging as a potential therapeutic target in TNBC, offering a new avenue for targeted therapies. FRα is overexpressed in a significant proportion of aggressive TNBC tumors.[146] Studies show FRα expression in 50% to over 70% of TNBC lesions, making it a relevant biomarker.[147] It is significantly higher in metastatic TNBC patients compared to those with early-stage disease. Therapeutic strategies followed for targeting FRα are (i) folic acid conjugation with NPs, (ii) CAR-T cell therapy, (iii) antibody-drug conjugates, (iv) monoclonal antibody, and (v) vaccine-based approaches. The clinical challenges associated with TNBC can be mitigated by identifying FRα expression in breast cancers and helping to identify target patients for anti-folate targeted therapy.

1.2.4. Wound Healing and Tissue Regeneration

In regular activities, every person is prone to wounds or injuries ranging from minor cuts to tissue damage or organ loss. In case of wounds or cuts, skin or fibroblast cells necessitate their actions and try to fill the gaps, which generally takes a longer time. Whereas, for organ loss or tissue damage, regeneration is the only alternative left for the patients. Further, some wounds are present over the outer layer of skin, whereas some are dipped by affecting underlying tissues and organs, which may be life-threatening in the long term. Therefore, wound care and tissue regeneration is a critical facet of healthcare therapeutics.

1.2.4.1. Global statistics on wound healing and tissue regeneration

Global statistics on wound healing and tissue regeneration indicate significant challenges and advancements in the biomedical field. Real-world data from the U.S. Wound Registry (USWR) reveals that most wounds do not heal as effectively as reported by many clinics. While

many centers claim healing rates are over 90% all over the world. Controlled trials show much lower healing rates, with average of around 40% for various types of wounds, including diabetic foot ulcers (DFUs) and venous leg ulcers (VLUs). The global wound care market was valued at approximately USD 23.15 billion in 2024 and is projected to grow at a compound annual growth rate (CAGR) of 4.19% from 2025 to 2030, driven by factors such as the increasing prevalence of chronic conditions like diabetes. It is expected to expand significantly in the Asia Pacific region, particularly in countries like China, where the incidence of chronic wounds such as DFUs is notably high, ranging from 17.03% to 42.84%. The mean healing rate for DFUs in clinical trials was approximately 37.9%, indicating a significant gap between reported outcomes and actual healing rates in controlled environments.[148-151]

1.2.4.2. Origin and categories of wounds

Wounds can originate from many events and can be broadly classified as acute and chronic wounds. Acute wounds heal quickly as compared to chronic wounds. Acute wounds are typically the result of sudden trauma, such as cuts, abrasions, or surgical incisions.[152, 153] Further, acute wounds can be either open (where the skin is broken) or closed (where the skin remains intact). Chronic wounds are commonly associated with underlying health conditions such as diabetes, venous insufficiency, or pressure ulcers. Chronic wounds typically do not show signs of healing within four weeks and may require more complex treatment approaches.[154] The distinction between acute and chronic wounds is crucial for determining appropriate care strategies and interventions. In turn of site of the wound, it can be categorized as internal or external wound. Internal wounds are mainly caused by compromised nervous and immune systems, decreased blood and oxygen delivery, etc. The factors that contribute to external wounds are penetrating (surgical, gunshot, and stabbing) and non-penetrating

(abrasion, contusion, and laceration) wounds.[153, 155] Thermal, electrical, chemical, or bites are considered under the miscellaneous category.

1.2.4.3. Phases of wound healing

Wound healing, crucial for tissue restoration, follows a sequence of overlapping phases: hemostasis, inflammation, proliferation, and remodeling (**Figure 1.3.**). Hemostasis occurs within minutes of injury, platelets aggregate at the injured site, changing shape and releasing chemical signals to initiate clotting. Fibrin is activated, forming a mesh that binds platelets, creating a clot to prevent further bleeding.[156-158] The second stage is the inflammation phase, where damaged cells, bacteria, and debris are cleared via phagocytosis. White blood cells engulf and destroy debris. Platelet-derived growth factors are released, promoting cell migration and causing division during the next phase.[159, 160] Further, the proliferation phase occurs, which is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization, wound contraction, and generation of vascular endothelial cells from new blood vessels. Fibroblasts grow and excrete collagen and fibronectin to form a new

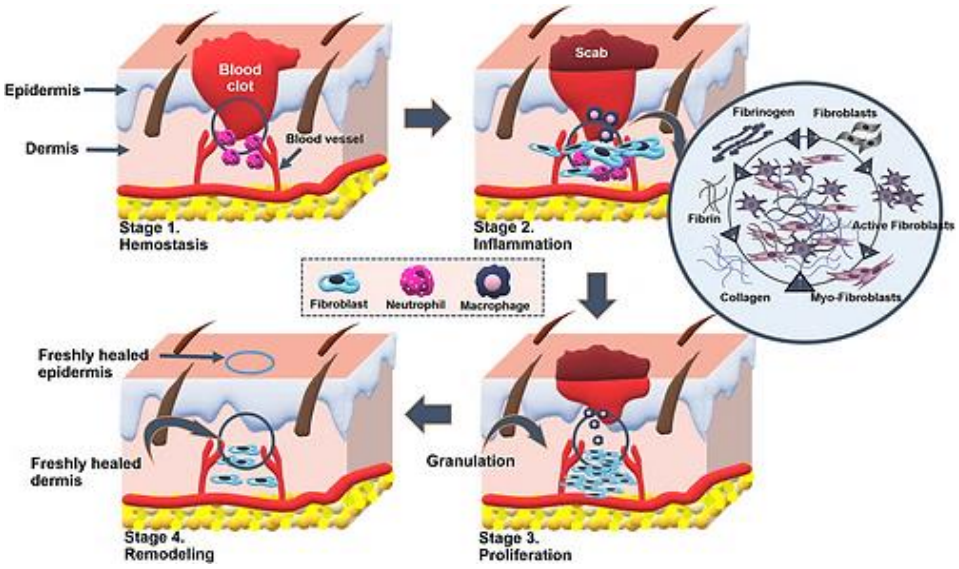

Figure 1.3. *Represents four stages of the wound healing process.[161]*

extracellular matrix. Epithelial cells proliferate and migrate across the wound bed, covering new tissue. Myofibroblasts decrease wound size by contracting the wound edges.[162, 163] Finally, in the remodeling phase, collagen is realigned along tension lines, unneeded cells are removed through apoptosis, and the tissue gets stronger compared to earlier days.[164] Optimal wound healing requires a balance of these phases and can be affected by factors such as blood supply, age, and underlying health conditions.

1.2.4.4. Polymers used for wound healing and tissue regeneration.

Both natural and synthetic polymers are widely used for wound healing and tissue regeneration. Polymers can offer multiple benefits such as adhesion, hydration, infection control, and hemostasis etc. Among various amino acids, glycine promotes wound healing by significantly enhancing collagen synthesis, formation of new blood vessels, the accumulation of fibroblasts, and helps in accelerating the restoration of damaged skin, etc.,[59] whereas phenylalanine's antioxidant and anti-inflammatory properties contribute in reducing inflammation and protecting cells, which aids in faster and improved wound repair.[165] Arginine supports wound repair by increasing nitric oxide production which promote angiogenesis and helps in supply of nutrients to the healing tissues.[166, 167] Proline helps in collagen formation and fibroblast function, thereby facilitating new tissue development and restoration. Lysine is essential for providing structural integrity to the skin and accelerating the repair process with improved healing quality and reduced scarring.[167] Histidine is used because of its unique peroxidase-mimic activity enables reactive oxygen species (ROS) generation for antibacterial effects. Alanine, especially as part of formulations with L-glutamine or as a component of peptides like carnosine, has been shown to promote nucleic acid and collagen biosynthesis, improving the integrity and tensile strength of healing tissues. Further, leucine is used in designing wound healing materials because leucine-based pseudo-proteins (LPPs) have been

shown to significantly enhance wound closure rates by modulating the cytokines production. A diverse range of polymers used for wound healing and tissue regeneration is listed in **Table 1.3**.

Table 1.3. List of polymers used for wound healing and tissue regeneration.

Polymer	Therapeutic agent	Types of use	Reference
L-nitroarginine-poly(ester amide)	-	Diabetic wound	[168]
Poly(ester amide) of lysine, arginine and phenylalanine	levofloxacin	Infected wound	[61]
Poly(ester amide) hydrogel conjugated with peptides	-	Infected wounds	[169]
PCL	Usnic acid	Full thickness wound	[170]
Chitosan/Gelatin	Ag NPs	Wound healing	[171]
Collagen/Hydroxy propyl methyl cellulose	Povidone Iodine	Regenerative tissue engineering	[172]
Collagen	Poly datin	Chronic wound	[173]
Poly ethylene glycol diacrylate/ catechol modified hyaluronic acid	Ag dopped silica NPs	Wound dressing	[174]
Poly(N-acryloyl glycine)	-	Acute wound	[59]
Poly(N-acryloyl glycine)	Sodium nitroprisside	Acute wound	[60]
Polyurethane/Chitosan	Linezolid	Diabetic wound	[175]
Bombyx mori silkworm-derived SF hydrogels	Fibroblast growth factor-1	Full-thickness skin excision	[176]
Fibrin-Chitin embedded Gelatin NPs based hydrogel	tigecycline	Cardiac surgery wounds	[177]
Poly(N-acryloylglycine)-co-(acrylamide)-co-(N-acryloyl-glutamate) hydrogel	-	Neuro regeneration	[178]

1.2.4.5. Toxicity associated with crosslinkers and chemically crosslinked copolymers

Crosslinkers and crosslinker-based copolymers present toxicity concerns in various applications. Certain crosslinkers like 1,4-butanediol diglycidyl ether (BDDE) have shown higher cytotoxicity, reactive oxygen species (ROS) production, and inflammatory responses compared to others like poly (ethylene glycol) diglycidyl ether (PEGDE) in cell lines.[[179] The balance between crosslinking of tissue and cell toxicity should be considered when developing a scaffold mimicking tissue with crosslinking strategies. Nanogels with higher flexibility due to less crosslinked matrices may interact better with cellular structures.[180]

Further, crosslinkers are highly chemically active and tend to bind with endothelial cells, body proteins, metal ions, enzymes, and lipids, which can hinder systemic cellular activities.[181] Based on all these limitations, a series of chemically synthesized covalently crosslinked NPs is restricted for clinical trials.[182] Thus, there is a huge scope to design a crosslinker free copolymer NPs for therapeutic applications specific for wound healing and tissue regeneration.

1.2.5. Angiogenesis

The process by which existing vasculature gives rise to new blood vessels is known as angiogenesis.[183, 184] Many pathological and physiological conditions, such as psoriasis, diabetic retinopathy, ischemic heart disease, rheumatoid arthritis, and tumor growth and metastasis, as well as embryonic development, wound healing, and tissue regeneration, depend on this process.[185] The angiogenesis can be of four types, (i) sprouting angiogenesis (endothelial cells proliferate from preexisting vessels to form new ones),[186, 187] (ii) vasculogenesis (blood vessels are created from endothelial precursor cells (angioblasts)), (iii) coalescent angiogenesis (entails capillary fusion to improve blood flow) and (iv) intussusceptive angiogenesis (happens when a vessel's wall divides it in half by extending into its lumen).[187, 188]

1.2.5.1. Polymers and amino acids-based nanoparticles: Their role in angiogenesis

The pro- or anti-angiogenic potential of polymers and amino acid-based NPs could be controlled by adjusting the synthesis and structural manipulation to complicated surface modifications, self-assembly, processing, and integration to make advanced materials. Targeting cancer therapy or regenerative application through NPs is an interesting field of research. The polymeric NPs or scaffolds can be used to load and deliver multiple drugs to

fight against cancer or to promote regeneration at the desired site.[189, 190] Among them, PEG, PLGA, PCL, PLA, chitosan, gelatin, heparin, and albumin have been extensively used for therapeutic angiogenesis, either in bare or modified form. A table has been given below with polymers used as carriers or scaffolds for therapeutic agents for angiogenesis (**Table 1.4**).

Table 1.4. List of polymer and scaffolds used for therapeutic applications.

Nanomaterials	Therapeutic agent/ Angiogenic Factor	Results	References
Chitosan	Ursolic Acid	Inhibition of angiogenesis in CAM and H22 xenograft model	[191]
Heparin	VEGF	Accelerated neovascularization and tissue repair	[192]
Poly(ethylene glycol)	-	support the lumen formation, expression of ECs proteins	[193]
Poly(lactic acid)	Paclitaxel	Involve in endocytosis and promote tube formation	[194]
Gelatin	bFGF	Improved capillary formation	[195]
Chitosan	SIKVAV Peptide	Increased expression of CD31	[196]
Hyaluronic Acid and Silk Fibroin	ZnO Nps	Accelerate wound closure and improved blood vessel formation	[197]
GelMA	Reduced graphene oxide	Improved in CAM model	[198]
Chitosan-Poly(ethylene oxide)/ PCL-Collagen	bFGF, EGF and silver sulfadiazine	Higher proliferation, attachment of fibroblasts and decreased inflammatory responses	[199]
PCL	Y_2O_3 NPs	Increased expression of VEGF, EGFR downregulation of TNF-α, and COX-2	[200]
Poly(*N*-acryloyl glycine)	-	Promote wound healing with tissue regeneration capacity	[60]
poly(*N*-acryloylglycine)-co-(acrylamide)-co-(*N*-acryloylglutamate)	-	Promote neuro regeneration	[178]
PLGA	Bevacizumab	Glioblastoma multiforme treatment	[201]
Chitosan	Raloxifene	In various breast cancer cell lines	[202]
PLGA	Aprepitant	Downregulation of VEGFA, VEGFB, and VEGFR-2	[203]

According to recent studies, the metabolism of amino acids is crucial for controlling angiogenesis and preserving vascular function. Endothelial cell (EC) migration, survival, and

proliferation all depend on amino acids, which are essential for angiogenesis.[204, 205] They also take part in the control of immunological responses, coagulation, fibrinolysis, vascular tone, redox homeostasis etc. In angiogenesis, glycine plays a complicated and conflicting role. It may inhibit angiogenesis in colorectal cancer by blunting VEGF-mediated EC proliferation, lowering the expression of inducible nitric oxide synthase (iNOS), and other mechanisms, according to some research.[206, 207] But according to other reports, it can stimulate angiogenesis by raising intracellular glycine levels in ECs through the glycine transporter 1 following VEGF stimulation.[204, 208] Cysteine is a necessary amino acid for corneal endothelial cells, protecting them from oxidative stress and promoting their growth and survival that improves endothelial function by raising nitrite levels and encouraging the synthesis of tetrahydrobiopterin.[205] Glutamine is metabolized to glutamate, which can stimulate endothelial cell germination. Analogs of proline can also promote the migration of endothelial cells. Tryptophan has been shown to promote cell proliferation and inhibit T-cell differentiation, thus promoting immune tolerance to cancer cells.[209] Further, Phenylalanine, regulates angiogenesis by maintaining mitochondrial function and modulating Notch/Wnt signaling pathways in endothelial cells through its mitochondrial phenylalanyl-tRNA synthetase (FARS2).[210]

1.2.5.2. Mechanism of angiogenesis induction

Angiogenesis induction is a multi-step, intricate process that involves numerous signaling pathways and factors. The key inducers of angiogenesis are hypoxia, growth factors, and extracellular matrix components. Hypoxia is a major cause of angiogenesis.[184, 187, 211] Pro-angiogenic factors are released by hypoxic cells to enhance oxygen delivery and encourage the formation of new blood vessels.[211, 212] Hypoxia-inducible factor 1-alpha (HIF-1α), a transcription factor that is essential for triggering the expression of pro-angiogenic genes such

as VEGF. It can be initiated and regulated by several growth factors.[213, 214] A strong pro-angiogenic factor, promotes the migration, proliferation, and survival of endothelial cells.[206, 215] Placental growth factor (PLGF) and VEGF-A, -B, -C, -D, and -E are among the members of the VEGF family which also promote angiogenesis. In addition, fibroblast growth factors (FGFs), platelet-derived growth factor (PDGF), angiopoietin, transforming growth factor (TGF), interleukins (IL), and tumor necrosis factor-α (TNF-α) are also pro-angiogenic factors.[186, 206] Proteases made by endothelial cells, such as matrix metalloproteinases (MMPs), break down the basement membrane that envelopes pre-existing vessels. Additionally, MMPs release angiogenic molecules such as FGFs and VEGFs.[214, 216]

At first, endothelial cells get activated by the key inducers. At the front edge of fresh sprouts, certain endothelial cells develop into tip cells.[216] VEGF gradients and other stimuli direct tip cells to extend filopodia, which are environmental sensors. Stalk cells multiply after tip cells to create the new vessel's body. Further, they migrate and get involved in lumen formation. Finally, PDGF and other factors recruit smooth muscle cells and pericytes to stabilize the new vessels.[213, 214] The signaling pathways involved here can be integrin signaling, notch signaling, VEGF/VEGFR, delta-notch, and WNTs[214, 215], and the metabolic factors responsible for blood vessel formation are glutamine metabolism, fatty acid oxidation (FAO), glycolysis[204], and amino acids (glycine, proline, and arginine)[207].

1.2.5.3. Models and methods used for accessing angiogenesis

Angiogenesis can be studied in both *in vitro* and *in vivo* models. *In vitro* models generally use endothelial cells to investigate angiogenesis. With these endothelial cells, varieties of assays such as tube formation assay, spheroid assay, migration assay, proliferation assay, cell adhesion, and permeability assay can be performed to establish the angiogenic nature of the designed NPs. Similarly, *in vivo*, models include organ culture assay, aortic ring assay, matrigel

plug assay, sponge implantation assay, mouse corneal angiogenesis assay, chick chorioallantoic membrane (CAM) assay, etc.[186, 217] Then, to visualize the changes in blood vessels, Intravital microscopy, Optical imaging, Dynamic contrast-enhanced (DCE) MRI, Ultrasound imaging, Microscopy, Radiotracer-based imaging, Angiography, Microcomputed tomography (micro CT), and Optical projection tomography like imaging techniques are used regularly.[188, 218] Further, to analyze the vascular structures and associated parameters, various software with imaging tools are used.[219]

1.2.5.4 Role of Image processing tool in angiogenesis over conventional methods

Numerous CAM assay studies employ diverse vasculature quantification methods, leading to outcome heterogeneity. These methods, like ImageJ, AngioTool, and Angioquant, each have limitations, including computational demands, manual steps, and long processing times, with associated software often being costly.[185, 219] While MATLAB-based tools like RAVE (Rapid Analysis of Vessel Elements) [220] and REAVER (Rapid Editable Analysis of Vessel Elements Routine) [221] emerged, RAVE lacks 3D image analysis capabilities, and REAVER remains under development. The absence of a standardized CAM vasculature quantification methodology limits inter-study data analysis and comparison. To address these limitations, we have used Image processing tool to fulfill the purpose. Gaussian adaptive threshold method followed by gray level co-occurrence matrix (GLCM)-based textural feature analysis for segmentation and quantitative angiogenesis assessment is performed.[222-224] Image processing tools can be easily integrated into a comprehensive image processing pipeline, including pre-processing steps (e.g., contrast enhancement, noise reduction) and post-processing steps on multiple images at once. Further, its handling capacity for uneven illumination, noise robustness, flexibility, and customization make the Image processing tool superior over other existing methods.[185] All the image analysis tools have its own advantages

and limitations which are listed in **Table 1.5**. Recently, machine learning and artificial intelligence-based techniques have also been developed to analyze angiogenesis, such as Q-VAT (Quantitative Vascular Analysis Tool),[224] BRAT (Batch Resourcing Angiogenesis Tool),[225] IKOSA, and NFA (network formation assay).[226]

Table 1.5. List of different angiogenesis software with its advantages and disadvantages.

Name of the Tool	Advantages	Disadvantages	References
Vessel Express	Quick and high-volume data analysis capacity	Shows error with specific antibodies	[225]
REAVER	High accuracy for segmentation	Under the development stage	[226]
RAVE	Able to differentiate between healthy and tumor cells	Lack of performing 3D analysis	[220, 227]
Angioquant	Specific for co-culture and CAM assay	A long time is needed	[228, 229]
WinFiber3D	Able to analyze specific orientation	-	[230, 231]
Amira	Able to remove noise and analyze 3D structures	Paid Software	[232]
AngioTool	Parameters can be adjusted based on the demand.	Require manual analysis, loose fine structures, lack of noise handling, and limited optimization options.	[233]
ImageJ	Code can be optimized based on the objective and can run on any operating system.	Requires a large amount of RAM, and some plugins allow for image manipulation	[217, 234-236]

1.3. MOTIVATIONS

1.3.1. Medical Needs and Clinical Challenges

Unmet Medical Needs: Many diseases, such as cancer, infectious diseases, and genetic disorders, lack effective treatments or have limited therapeutic options. Conventional treatments often have severe side effects, toxicity, or limited efficacy, highlighting the need for safer and more effective alternatives. Current drug delivery systems may not effectively target the desired site of action, leading to reduced efficacy and increased toxicity.

Clinical Challenges: Cancer treatment often involves non-specific targeting, which damages healthy tissues and results in limited efficacy. The rise of cancer-resistant, antibiotic-resistant, and the need for more effective treatments for various cancers (TNBC) and infectious diseases have driven the development of new therapeutic agents. Further, the need for biomaterials to promote tissue regeneration and repair drives the development of amino acid-based copolymers.

We aim to design amino acid-based copolymers that offer biocompatibility, biodegradability, and non-toxicity, addressing the concerns associated with conventional materials. They can be designed to target specific cells/ tissue or engineered to promote tissue regeneration, repair, and replacement.

1.3.2. Customization and Tailoring

The option to tailor mechanical properties, customize degradation rate, and tune release profile may assist us in developing more versatile amino acid-based copolymer NPs. These customization and tailoring helped in achieving targeted therapies with cell-specific interaction and tissue-specific regeneration properties. The advantages of customization and tailoring include improved efficacy, reduced side effects and enhanced patient safety.

1.3.3. Sustainability Consideration

Sustainability considerations drive the development of amino acid-based copolymers by emphasizing biodegradability, eco-friendly sourcing, reduced toxicity, alignment with green chemistry principles, and responsible approaches to addressing clinical needs. These factors collectively highlight the potential of these copolymer materials to contribute positively to both human health and environmental sustainability. By addressing economic concerns like cost-effective production, reduction in waste management cost, and job creation; and social burdens

such as increased accessibility and patient safety, by which we can create innovative solutions. Moreover, these amino acid-based copolymers support a circular economy by enabling material recycling and degradation into non-toxic natural metabolites, further minimizing environmental impact and fostering long-term ecological balance.

1.3.4. Regulatory Support and Market Demand

Regulatory Support: Government agencies are providing research grants and funding to support the development of innovative biomaterials, including amino acid-based copolymers. Regulatory agencies offer fast-track approval pathways, priority review, and orphan drug designation for biomedical products that address unmet medical needs. International regulatory agencies are working together to harmonize regulatory requirements, facilitating the global development and approval of biomedical products.

Market Demand: The global nanomedicine market is projected to reach $690.96 billion by 2029, growing at a CAGR of 16.2%. Particularly, the cancer nanomedicine market alone is expected to reach $350 billion by 2025, indicating robust growth driven by increasing chronic diseases.[89] Further, the growing interest in personalized medicine and targeted therapies drives the demand for biomaterials that can be tailored to specific patient needs.

Thus, understanding the intersection of regulatory support and market demands helps us to build collaborations between industry, academia, and regulatory agencies.

1.3.5. Scientific Advancement

Research on amino acid-based copolymers contributes to a deeper understanding of biomaterials, their properties, and interactions with living tissues. Developing amino acid-based copolymers enables the creation of novel polymer architectures, which can lead to breakthroughs in materials science and biotechnology. Additionally, expertise from multiple

interdisciplinary fields such as materials science, biology, chemistry, and medicine can work together to bring innovation and advancement in science. Furthermore, ethical considerations ensure that the development and application of amino acid-based copolymers prioritize patient safety, informed consent, equitable access, and responsible use of biological resources to uphold societal trust and integrity in biomedical innovation.

1.3.6. Ethical Consideration

Ethical considerations emphasize the importance of informed consent, where patients should be aware of the materials used in their treatments. Amino acid-based copolymers, being derived from natural sources, may provide a more transparent option for patients concerned about synthetic chemicals. Further, the development of amino acid-based copolymers can reduce the need for animal testing, as these materials can be designed to mimic human tissues and organs. These copolymers can be engineered to minimize risk and maximize benefit to human subjects, ensuring that research participants are protected and respected. Our ultimate goal is to transform lab-scale products into clinical treatment options by minimalizing the use of animal models in the long run.

1.4. OBJECTIVES WITH JUSTIFICATIONS

Based on the above research gaps, this work "*Development of Amino acid-based Copolymeric NPs for Organ Targeting Delivery of Drugs and Tissue Regeneration*" is divided into four major objectives as mentioned below:

Objective 1: Organ Targeting Drug Delivery Systems (OTDDS) of poly[(*N*-acryloyl glycine)-*co*-(*N*-acryloyl-*L*-phenylalanine methyl ester)] Copolymer Library and Effective treatment of Triple Negative Breast Cancer

Justification: This objective aims to design a library of copolymers, i.e., p(NAG-*co*-NAPA)$_{(x:y)}$ by varying the concentration of mers, which have different extents of targeting efficiencies to different organs. Based on the treatment requirement, any of the NPs can be considered to achieve maximum therapeutic efficiencies which may not be restricted to major organs like the breast, kidney, liver, heart, and lungs.[94, 138, 237] Further, based on the obtained results, as a proof-of-concept, p(NAG-*co*-NAPA)$_{(1:4)}$ was potentiated with anticancer drugs for TNBC treatment.

Objective 2: Co-delivery of Dihydroartemisinin and Piperin by Folate decorated poly[(*N*-acryloyl glycine)$_1$-*co*-(*N*-acryloyl-*L*-phenylalanine methyl ester)$_4$] Copolymer for Triple Negative Breast Cancer Treatment

Justification: This objective aims to extend the work established in objective 1 by modifying the p(NAG-*co*-NAPA)$_{(1:4)}$ NPs. The hypothesis is that by conjugating p(NAG-*co*-NAPA)$_{(1:4)}$ NPs with folic acid, the targeting efficiencies towards TNBC can be enhanced.[141-143] Further, a mechanistic pathway has been established for the inhibition of TNBC by these developed nanoformulations for future clinical trials.

Objective 3: Self-assembled Amino-based copolymer NPs for Wound Healing and tissue regeneration: structure studied through Molecular Dynamics Simulation

Justification**:** The objective is based on the synthesis of crosslinker free and biocompatible copolymer p(NAG-*co*-NAPA)$_{wc}$ NPs for wound healing and tissue regeneration applications. Although crosslinker-based copolymer NPs offer promising advantages in terms of stability and targeted drug delivery, several issues need to be addressed to optimize their performance. These include ensuring stability against dissociation, minimizing intermicellar crosslinking, assessing biocompatibility, controlling drug release kinetics, simplifying synthesis protocols, etc.[182, 238, 239]

Objective 4: Angiogenic properties of Poly(N-acryloyl-glycine)-*co*-(N-acryloyl-L-phenylalanine methyl ester) nanoparticles: Quantitative Assessment via Gray Level Co-occurrence Matrix based Image Processing

Justification: The objective is to develop a new method of assessing angiogenesis using gray-level co-occurrence matrix (GLCM)-based texture features image processing tool, which could be co-related with more reliable parameters. To achieve this, 1:1 ratio based *N*-acryloyl glycine and *N*-acryloyl-*L*-phenylalanine methyl ester, i.e., p(NAG-*co*-NAPA) copolymer NPs has been synthesized which is biocompatible and angiogenic. The pro-angiogenic action of NPs was assessed through 'tube formation assay' and '*in ovo'* model by using the Gray Level Co-occurrence Matrix (GLCM). As among the various textural features, accounting for both spatial and intensity information, it has shown to be capable of analyzing angiogenesis as well as to predict therapeutic doses for developed p(NAG-*co*-NAPA) NPs for biomedical applications. However, compared to conventional tools (such as Angiotool), the image processing method is fully automated where no manual segmentation and noise removal steps are required.[68, 240, 241]

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